Herbicide detoxification by glutathione *S*-transferases as implicated from X-ray structures†

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Abstract: Herbicide selectivity is a major factor in agricultural weed control and results from the differential detoxification ability of plant species. The special agronomic value of plant GSTs originates mainly from their metabolic herbicide detoxification properties that enhance the herbicide tolerance of crops. Glutathione *S*-transferases (GSTs) are a ubiquitous family of multifunctional enzymes involved in the metabolization of a broad variety of xenobiotics (eg herbicides in plants) and reactive endogenous compounds through covalent linkage to glutathione. The metabolization of FOE 5043 results in a GSH conjugate that is subsequently degraded by release of the thiadiazole moiety. The comparison of crystal structures of different GST classes, including plant GSTs, provides a model system to understand active-site interactions on a molecular level. Additional protein structures of three plant GSTs (*Arabidopsis thaliana* GST, GST I and III from *Zea mays var. mutin*) in complex with several ligands (*S*-hexylglutathione, *S*-lactoylglutathione, and FOE 5043) may be tools to supply detailed knowledge for the rational design of new herbicides and GSTs for selectivity optimization in crops.

Keywords: glutathione *S*-transferase; crystallography; plants; herbicide detoxification; protein structure, ligand binding

1. INTRODUCTION

Herbicide treatment of crops increases agricultural productivity through economic weed control. The use of herbicides is based on their selectivity, which mostly results from the differential detoxification ability of different plant species.[1,2] Glutathione *S*-transferases (GSTs) – a family of multifunctional enzymes – provide a general enzymatic system of metabolic detoxification in higher plant cells. These ubiquitous enzymes, which have also been found in all vertebrates, insects, nematodes, yeasts, and aerobic bacteria, catalyze the electrophilic addition of the reduced form of the tripeptide glutathione (γ-Glu-Cys-Gly) to a variety of hydrophobic compounds.[3,4] The resulting glutathionyl-*S* conjugates are less reactive and more polar than the metabolized exogenous chemicals (xenobiotics) or reactive endogenous substrates. In contrast to animals, where the conjugates are catabolized and excreted,[5] plants have to store the soluble glutathione *S*-conjugates in the vacuole, because of the lack of excretion pathways.[6] As GSTs play a key role in the cellular detoxification metabolism and the development of resistance towards carcinogens, drugs and pesticides in different organisms,[7,8] the isozyme multiplicity and physiological role of different GSTs have been studied in great detail.[9–12] The soluble cytosolic GSTs are classified according to sequence similarities and immunological cross-reactivity into Alpha, Mu, Pi Sigma and Theta classes.[13] The X-ray structures of all five GST classes have been determined, showing the similar dimeric topology of all GSTs.[14–18] Each sub-unit contains a kinetically independent active site[19] with a conserved tyrosine residue, involved in catalysis. Interestingly, in theta-class GSTs (eg plant GSTs) there is a serine that acts as catalytic residue.[20] Here we present an overview comprising the so-far-solved protein structures of GSTs and GST-ligand complexes, which provide a detailed picture of the key interactions within the active site of these protective and detoxifying enzymes. Especially the three-dimensional structure

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of plant GSTs[20] and data on herbicide binding (FOE 5043)[21] may together serve as a first model for the deeper understanding of the herbicide detoxification system in plants on a molecular level. As for a variety of herbicides the major resistance and selectivity factor in plants is the conjugation of glutathione, these data may also serve to rationalize aspects concerning the mechanism of herbicide tolerance in crops.

2 PLANT GLUTATHIONE *S*-TRANSFERASES

Plant GSTs have been studied intensively with regard to their ability to metabolize xenobiotics and natural compounds by GSH conjugation. The first GST activity reported in plants was found in maize,[22] where it is involved in atrazine detoxification. This ability to metabolize atrazine has been shown to be the major resistance factor for this herbicide in maize.[23] Six GST isoforms have been characterized so far in maize, differing in their regulation and substrate specificities.

All known plant GSTs are considered to belong to the theta class – also named 'default class' – which exhibits the widest distribution among species[24] and comprises phylogenetic old GSTs with very heterogenous primary sequences. An additional classification into sub-classes based on amino acid sequence identity and intron : exon conservation was proposed by Droog *et al.*[25,26] Following this scheme, the plant isoenzymes are divided into type I GSTs, which take part in herbicide detoxification, the ethylene-regulated type II enzymes from carnation, and the auxin-regulated type III proteins. As type III isozymes seem to be unique to plants a new GST class named tau was suggested by Droog.[27] The isoforms termed GST I, III and IV are dimers composed of GST29, GST26, and GST27 sub-units (numbers indicate molecular mass), whereas GST II is a dimer consisting of GST29 and GST27.[28–31]

GST I and III are expressed constitutively in maize, whereas GST II and IV represent safener-induced enzymes.[25] A sequence alignment of four selected plant GSTs is presented in Table 1. Recently auxin-regulated gene expression products which are apparently related to numerous GST functions in plants have been detected.[27] The number of plant GST sequences is growing and comprises at present 35 partially or fully sequenced genes encoding GST isozymes from 13 plant species, demonstrating the importance of GST activities in plants.

3 HERBICIDE RESISTANCE AND TISSUE PROTECTION IN PLANTS

Herbicides are designed to affect crucial biological processes in plants such as photosynthesis (atrazine) and biosynthesis of essential amino acids (chlorsulfuron). As these processes are common to both crop and weed, a selectivity criterion is needed. This can be defined as differential herbicide uptake between weed and crop, since it is known that plants are able to metabolize herbicides by a variety of enzymatic reactions. Herbicide resistance in crops that is not due to changed target sites is mostly attributed to enhanced detoxification properties. Detoxification by GSH conjugation is a well-documented, highly effective system in higher plants that is able to metabolize various herbicides such as triazines, thiocarbamates and chloroacetanil-

Table 1. Amino acid sequences of four selected plant glutathion *S*-transferases: maize GST type-I (GST I);[31] maize GST type-III (GST III);[45] potato GST type-III (prpGST)[47] and *Arabidopsis thaliana* GST type-I (araGST).[48]

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          1                                                  50
GST I      APMKLYGAV MSWNLTRCAT ALEEAGSDYE IVPINFATAE HKSPEHLVRN
GST III   MAPLKLYGMP LSPNVVRVAT VLNEKGLDFE IVPVDLTTGA HKQPDFLALN
ara GST   MAGIKVFGHP ASIATRRVLI ALHEKNLDFE LVHVELKDGE HKKEPFLSRN
prp GST   MAEVKLLGLR YSPFSHRVEW ALKIKGVKYE FIEEDL...Q NKSPLLLQSN
          51                                                 100
GST I     PF.GQVPALQ DGDLYLFESR AICKYAARKN KPE...LLRE G..NLEEAAM
GST III   PF.GQIPALV DGDEVLFESR AINRYIASKY ASEGTDLLPA T....ASAAK
ara GST   PF.GQVPAFE DGDLKLFESR AITQYIAHRY ENQGTNLLQT DSKNISQYAI
prp GST   PIHKKIPVLI HNGKCICESM VILEYIDEAF ..EGPSILPK DPYDRALARF
          101                                                150
GST I     VDVWIEVEAN QYTAALNPIL FQVLISPMLG GTTDQKVVDE NLEKLKKVLE
GST III   LEVWLEVESH HFHPNASPLV FQLLVRPLLG GAPDAAVVEK HAEQLAKVLD
ara GST   MAIGMQVEDH QFDPVASKLA FEQIFKSIYG LTTDEAVVAE EEAKLAKVLD
prp GST   WAKYVEDKG. .......... .AAVWKSFFS KGEEQEKAKE EAYEMLKILD
          151                                                200
GST I     VYEARLTKCK YLAGDFLSLA DLNHVSVTLC L..FATPYAS VLDA...YPH
GST III   VYEAHLARNK YLAGDEFTLA DANHA..LLP A..LTSAPPR PGCVAAR.PH
ara GST   VYEARLKEFK YLAGETFTLT DLHHIPAIQY L..LGTPTKK LFTE...RPR
prp GST   ...NEFKDKK CFVGDKFGFA DIVANGAALY LGILEEVSGI VLATSEKFPN
          201                                  240
GST I     VKAWWSG... .....LMERP SVQK.VAALM KPSA
GST III   VKAWWEA... .....IAARP AFQKTVAAIP LPPPPSSSA
ara GST   VNEWVAE... .....ITKRP ASEKVQ
prp GST   FCAWRDEYCT QNEEYFPSRD ELLIRYRAYI QPVDASK
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ides.[13,32,33] According to Jacoby & Ziegler[34] the detoxification process can be divided into three phases. In the first phase (transformation) functional groups are introduced enzymatically onto the substrate. During the second phase (conjugation) a conjugate between the activated substrate and GSH is formed catalytically by GSH, leading to a less toxic and more soluble compound. In the third phase (compartmentalization) the conjugate is transferred through active transport by a 'glutathione pump' into the vacuole of the plant cell. Following this scheme, the GST can be regarded as phase II enzyme. Plant GSTs not only inactivate toxic compounds through conjugation, but also exhibit a number of additional functions, playing an important role in the plant defence system. GSTs are involved in the inactivation of cytotoxic plant metabolites and in stress responses induced by pathogenic attack, oxidative stress and phytohormone treatment. In addition to their enzymatic function some GSTs can also serve as ligand-binding proteins (ligandin function), thus facilitating the intracellular transport and storage of hydrophobic non-substrate compounds, such as metabolites, drugs and hormones.[35,36] Additionally, the GSTs play a central role in the cellular auxin response. As auxins (generic term for shoot-elongation-inducing agents) both bind to several plant GST proteins and induce GST gene expression,[37,38] a direct interaction among at least some of them seems to be clear.

The data also indicate that the binding of different auxins to GSTs occurs at different catalytic and non-catalytic sites.[39] The role of the GST auxin-receptor function may be the regulation of activity through GSH conjugation and transport within cells. Pathogen attack and stress-inducing agents (eg heavy metals, ethylene, ozone) induce plant GST gene expression[40,41] through formation of active oxygen species such as peroxide ('oxidative burst').[42] The increased GST level in turn protects cellular components. Interestingly, peroxide not only stimulates the transcription of GSTs but also of 'protective' glutathione peroxidases.[43] In order to prevent the excessive accumulation of phytotoxic secondary metabolites, microbial toxins and agrochemicals within the cell, plant GSTs function as non-enzymatic carrier proteins (ligandins) to eliminate those compounds by transporting them into the vacuole (storage excretion).[35]

4 PLANT GST PROTEIN STRUCTURES AND COMPARISON TO OTHER KNOWN CRYSTAL STRUCTURES OF THE GST FAMILY

The X-ray structures of a number of class alpha, mu, pi, and theta GST isozymes were solved, and although each class exhibits characteristic differences, particular at the catalytic centre and the C-terminus, the overall topologies were found to be very similar.[12, 20] The first plant GST structure (*Arabidopsis thaliana* Heynh) was elucidated by Reinemer *et al*,[20] but recently structures of maize GST type III and type I (type I in complex with lactoylglutathione) have also been solved.[44,45] Glutathione *S*-transferase from *A. thaliana* (araGST) forms a homodimer with typical type I plant GST topology. Each sub-unit shows a modular arrangement consisting of two spatially distinct domains connected by a linker segment of different length (eg araGST: 15 residues). The *N*-terminal, smaller domain (domain I) exhibits an α/β-structure with a central, four-stranded and left-hand twisted β-sheet flanked by α-helices and 3_{10}-helices. A characteristic cis-proline bond (Pro55 in araGST), observed in all three plant GST structures,[20,44,45] is situated in a conserved region and is crucial for correct folding of the glutathione binding site. The larger C-terminal domain II is completely α-helical and consists of six amphipathic helices forming a right-handed spiral. The globular GST homodimer forms a prominent large cleft between domains I and II, where the catalytic centres are located. There is one kinetically independent active site per sub-unit,[4] which is composed of two distinct subsites, G-site and H-site. Glutathione binding occurs in the hydrophilic and highly specific G-site, whereas the adjacent hydrophobic H-site pocket promotes binding of structurally diverse substrates with lower specificity. Interestingly, two molecules of hexylglutathione are found per sub-unit active site in the araGST structure. One molecule occupies the G- and H-sites as expected, whereas the second molecule of hexylglutathione is associated only through weak interactions, in a cleft (active site) with preference for spacious, hydrophobic ligands. Although araGST and mammalian GSTs share significant structural homology that is underlined by the resemblance of orientation and conformation of the bound *S*-hexylglutathione in araGST and human GST P1-1,[20,46] they differ in the C-terminal region, where a broader and deeper H-sub-site in plant GST (*A. thaliana*) is observed. All three plant GSTs[20,44,45] exhibit the GST typical structure: they are homodimers consisting of two distinct domains. The comparison of these plant GST structures reveals structural differences mainly located at the hydrophobic substrate binding site (H-site), the linker segment, and the C-terminal region. On the basis of the comparison of the maize GST I /lactoylglutathione complex structure with the apo maize GST III structure a ligand-induced conformational change of a ten-residue loop was suggested.[44] Plate 1 shows the araGST dimer in complex with the bound *S*-hexylglutathione. In general, residues involved in glutathione binding within the G-site of all observed GST structures are conserved or conservatively replaced, whereas the variations concerning the H-site are much larger and constitute an important part of the structural diversity of the compared GSTs. Evidence has been provided that a conserved tyrosine residue close to the

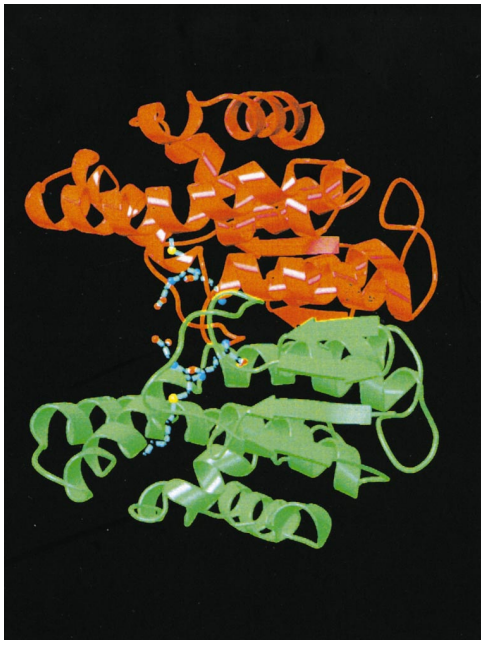

Plate 1. The araGSTdimer in complex with the bound ligand *S*-hexylglutathione presented as a ribbon model. The monomers are coloured in red and green, respectively.

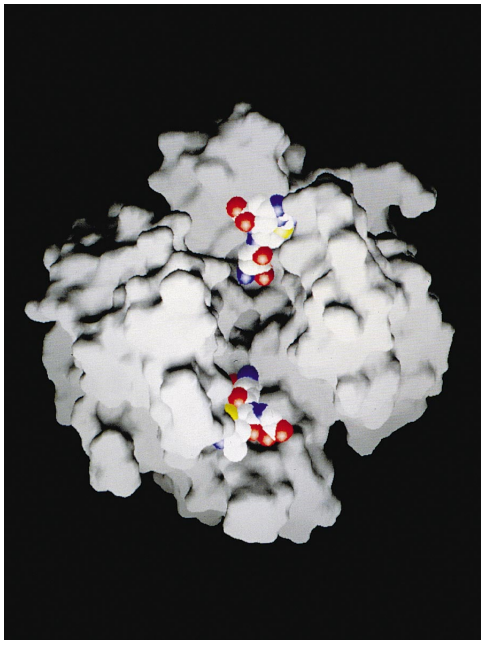

Plate 2. Surface representation of araGST with FOE-glutathione conjugates bound to the active sites of the dimeric protein. The catalytic centres are located directly at the dimer interface of the protein molecule.

N-terminus is responsible for catalytic activity through activation of the GSH thiol group[11] in four classes of GSTs. As araGST contains a glycine that is clearly not involved in catalysis,[20] a conserved serine, (araGST, Ser11) positioned closer to the C-terminus is suggested to take over the catalytic role in theta class GSTs.[12,20] The structural comparison of different plant GSTs involved in herbicide metabolism may provide a basis to manipulate herbicide tolerance, which can possibly be extended by rational design of new herbicides and GSTs on a molecular level.

5 INTERACTIONS OF FOE 5043 HERBICIDE WITH araGST

Herbicide tolerance at certain application rates of FOE 5043 [*N*-(4-fluorophenyl)-*N*-isopropyloxyacetamide] is observed in maize, soybean, wheat and other crops due to metabolization of the active ingredient. Detoxification rates in plant tissue could be studied by the use of ^{14}C-labelled herbicide and revealed glutathione conjugation as the main metabolite and main contribution to FOE 5043 tolerance in maize, wheat and soya.[21] Furthermore, FOE 5043 glutathione conjugates were isolated from maize, exploiting the increased water solubility of the glutathione conjugates compared to the active agrochemical.[21] The reaction product of FOE 5043 and GSH was enzymatically produced *in vitro* utilizing recombinant maize GST. The chemical structure of the in-vitro reaction product of the GST detoxification process was determined by NMR spectroscopy and revealed the nucleophilic attack of the GSH thiol group, causing irreversible degradation of the thiadiazole group of FOE 5043. Plate 2 illustrates the binding of FOE-glutathione conjugate to araGST.

REFERENCES

1 Cole DJ, Detoxification and activation of agrochemicals in plants. *Pestic Sci* **42**:209–222 (1994).

2 Lamoureux GL, Shimabukuru RH and Frear DS, Glutathione and glucoside conjugation in herbicide selectivity, in *Herbicide Resistance in Weeds and Crops*, ed by Caseley JC, Cussans GW and Atkin RK, Butterworth-Heinemann, Oxford, UK. pp 227–261 (1991).

3 Rushmore TH and Pickett CB, Glutathione *S*-transferases: Structure, regulation, and therapeutic implications. *J Biol Chem* **268**:11475–11478 (1993).

4 Mannervik B and Danielson UH, Glutathione transferases: Structure and catalytic activity. *CRC Crit Rev Biochem* **23**:283–337 (1988).

5 Boyland E and Chasseaud LF, The role of glutathione and glutathione *S*-transferase in mercapturic acid biosynthesis. *Adv Enzymol* **32**:173–219 (1969).

6 Sandermann HJ, Plant metabolism of xenobiotics. *Trends Biochem Sci* **17**:82–84 (1992).

7 Hayes JD and Wolf CR, Role of glutathione transferase in drug resistance, in *Glutathione Conjugation: Mechanisms and Biological Significance*, ed by Sies H and Ketterer B, Academic Press, London, UK. pp 316–356 (1988).

8 Tsuchida S and Sato K, Glutathione *S*-transferases and cancer. *CRC Crit Rev Biochem Mol Biol* **27**:337–384 (1992).

9 Marrs KA, The function and regulation of glutathione *S*-transferases in plants. *Annu Rev Plant Physiol Plant Mol Biol* **47**:127–158 (1996).

10 Dirr HW, Reinemer P and Huber R, X-ray crystal structure of cytosolic glutathione *S*-transferases. Implications for protein architecture, substrate recognition and catalytic function. *Eur J Biochem* **220**:645–661 (1994).

11 Armstrong RN, Glutathione *S*-transferases: structure and mechanism of an archetypical detoxification enzyme. *Adv Enzymol* **69**:1–44 (1993).

12 Wilce MC, Board PG, Feil SC and Parker MW, Crystal structure of a theta-class glutathione transferase. *EMBO J* **14**:2133–2143 (1995).

13 Leavitt JRC and Penner D, In-vitro conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. *J Agric Food Chem* **27**:533–536 (1979).

14 Sinning I, Kleywegt GJ, Cowan SW, Reinemer P, Dirr HW, Huber R, Gilliland GL, Armstrong RN, Ji X, Board PG, Olin B, Mannervik B and Jones TA, Structure determination and refinement of human alpha class glutathione transferase A1-1, and a comparison with the mu and pi class enzymes. *J Mol Biol* **232**:192–212 (1993).

15 Raghunathan S, Chandross RJ, Kretsinger RH, Allison TJ, Penington CJ and Rule GS, Crystal structure of human class mu glutathione transferase GSTM2-2. Effects of lattice packing on heterogeneity. *J Mol Biol* **238**:815–832 (1994).

16 Reinemer P, Dirr HW, Ladenstein R, Schäffer J, Gallay O and Huber R, The three-dimensional structure of class II glutathione *S*-transferase in complex with glutathione sulfonate at 2.3 Å resolution. *EMBO J* **10**:1997–2005 (1991).

17 Ji X, Zhang P, Armstrong RN and Gilliland GL, The three-dimensional structure of a glutathione *S*-transferase from the mu gene class. Structural analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2 Å resolution. *Biochemistry* **31**:10169–10184 (1992).

18 García-Sáez I, Parraga A, Phillips M, Mantle TJ and Coll M, Molecular structure at 1.8 Å of mouse liver pi glutathione *S*-transferase complexed with *S*-(*p*-nitrobenzyl)glutathione and other inhibitors. *J Mol Biol* **237**:298–314 (1994).

19 Danielson UH and Mannervik B, Kinetic independence of the subunits of cytosolic glutathione transferase from the rat. *Biochem J* **231**:263–267 (1985).

20 Reinemer P, Prade L, Hof P, Neuefeind T, Huber R, Zettl R, Palme KJS, Koelln I, Bartunik HD and Bieseler B, Three-dimensional structure of glutathione *S*-transferase from *Arabidopsis thaliana* at 2.2 Å resolution: Structural characterization of herbicide-conjugating plant glutathione *S*-transferases and a novel active site architecture. *J Mol Biol* **255**:289–309 (1996).

21 Bieseler B, Fedke C, Neuefeind T, Etzel W, Prade I and Reinemer P, Maize selectivity of FOE 5043: Degradation of active ingredient by glutathione-*S*-transferases. *Pflanzenschutz-Nachrichten Bayer* **50**:117–140 (1997).

22 Frear DS and Swanson HR, Biosynthesis of *S*-(4-ethylamino-6-isopropylamino-2-*s*-triazino)glutathione: partial purification and properties of glutathione *S*-transferase from corn. *Phytochemistry* **9**:2123–2132 (1970).

23 Shimabukuro, RH, Swanson HR and Walsh WC, Glutathione conjugation: atrazine detoxification mechanism in corn. *Plant Physiol* **46**:103–107 (1970).

24 Buetler TM and Eaton DL, Glutathione *S*-transferase: amino acid sequence comparison, classification and phylogenetic relationship. *Environ Carcino Ecotox Rev* **C10**:181–203 (1992).

25 Droog FJN, Hooykaas PJJ, Libbenga KR and van der Zaal EJ, Proteins encoded by an auxin regulated gene family of tobacco share limited but significant homology with glutathione *S*-transferases and one member indeed shows in-vitro GST activity. *Plant Mol Biol* **21**:965–972 (1993).

26 Droog FJN, Hooykaas PJJ and van der Zaal BJ, 2,4-Dichlorophenoxyacetic acid and related chlorinated compounds inhibit two auxin-related type-III tobacco glutathione *S*-transferases. *Plant Physiol* **107**:1139–1146 (1995).
27 Droog F, Plant glutathione *S*-transferases, a tale of theta and tau. *J Plant Growth Regul* **16**:95–107 (1997).
28 Grove G, Zarlengo RP, Timmerman KP, Li N, Tam MF and Tu C, Characterization and heterospecific expression of cDNA clones of genes in the maize GSH *S*-transferase multigene family. *Nucleic Acid Res* **16**:425–438 (1988).
29 Irzyk G, Potter S, Ward E and Fuerst EP, A cDNA clone encoding the 27-kiloDalton subunits of glutathione *S*-transferase IV from Zea mays. *Plant Physiol* **107**:311–312 (1995).
30 Jepson I, Lay VJ, Holt DC, Bright SWJ and Greenland AJ, Cloning and characterization of maize herbicide safener-induced cDNAs encoding subunits of glutathione *S*-transferase isoforms I, II and IV. *Plant Mol Biol* **26**:1855–1866 (1994).
31 Shah DM, Hironaka CM, Wiegand RC, Harding EI, Krivi GG and Tiemeier DC, Structural analysis of a maize gene coding for glutathione *S*-transferase involved in herbicide detoxification. *Plant Mol Biol* **6**:203–211 (1986).
32 Ezra G and Stephenson GR, Comparative metabolism of atrazine and EPTC in proso millet (*Panicum miliaceum* L.) and corn. *Pestic Biochem Physiol* **24**:207–212 (1985).
33 Komvies AV, Komvies T and Dutka F, Effects of herbicides on the activity of glutathione *S*-transferases in maize. *Cereal Res Commun* **13**:253–257 (1985).
34 Jakoby WB and Ziegler DM, The enzymes of detoxification. *J Biol Chem* **265**:20715–20718 (1990).
35 Listowski L, Abramovitz M, Homma H and Niitsu Y, Intracellular binding and transport of hormones and xenobiotics by glutathione *S*-transferases. *Drug Metab Rev* **19**:305–318 (1988).
36 Listowski L, Glutathione *S*-transferases: intracellular binding, detoxification, and adaptive responses, in *Hepatic Transport and Bile Secretion: Physiology and Pathophysiology*, ed by Tavolini N and Beck PD, Raven Press, New York, USA. pp 397–405 (1993).
37 Takahashi Y, Ishida S and Nagata T, Auxin-regulated genes. *Plant Cell Physiol* **36**:383–390 (1995).
38 Jones AM, Auxin-binding proteins. *Annu Rev Plant Physiol Plant Mol Biol* **45**:393–420 (1994).
39 Bilang J, Macdonald H, King PJ and Sturm A, A soluble auxin-binding protein from *Hyoscyamus muticus* is a glutathione *S*-transferase. *Plant Physiol* **102**:29–34 (1993).
40 Thenaken R, Levine A, Brisson LF, Dixon RA and Lamb C, Function of the oxidative burst in hypersensitive disease resistance. *Proc Natl Acad Sci USA* **92**:4158–4163 (1995).
41 Ulmasov T, Ohimiya A, Hagen G and Guilfoyle T, The soybean GH2/4 gene that encodes a glutathione *S*-transferase has a promoter that is activated by a wide range of chemical agents. *Plant Physiol* **108**:919–998 (1995).
42 Levine A, Tenhaken R, Dixon R and Lamb C, Hydrogen peroxide from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**:583–593 (1994).
43 Ballas N, Wong LM and Theologis A, Identification of the auxin-responsive element (AuxrRE) in the primary indole acetic acid inducible gene PS-IAA4/5 of pea (*Pisum sativum*). *J Mol Biol* **233**:580–596 (1993).
44 Neuefeind T, Huber R, Dasenbrock H, Prade L and Bieseler B, Crystal structure of herbicide-detoxifying maize glutathione *S*-transferase-I in complex with lactoylglutathione: Evidence for an induced-fit mechanism. *J Mol Biol* **274**:446–453 (1997).
45 Neuefeind T, Huber RPR, Knäblein J, Prade L, Mann K and Bieseler B, Cloning, sequencing, crystallization and X-ray structure of glutathione *S*-transferase-III from *Zea mays var. mutin*: A leading enzyme in detoxification of maize herbicides. *J Mol Biol* **274**:577–587 (1997).
46 Reinemer P, Dirr HW, Ladenstein R, Huber R, Lo Bello M, Federici G and Parker MW, Three-dimensional structure of class II glutathione *S*-transferase from human placenta in complex with *S*-hexylglutathione at 2.8 Å resolution. *J Mol Biol* **227**:214–226 (1992).
47 Hahn K and Strittmatter G, Pathogen-defence gene prp1-1 from potato encodes an auxin-responsive glutathione *S*-transferase. *Eur J Biochem* **226**:619–626 (1994).
48 Zettl R, Schell J and Palme K, Photoaffinity labelling of *Arabidopsis thaliana* plasma membrane vesicles by 5-azido-(7-3*H*)indole-3-acetic acid: identification of a glutathione *S*-transferase. *Proc Natl Acad Sci USA* **91**:689–693 (1994).